PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2018.677.682

Research Article
Using Molasses in a Rice Straw Urea Lime Molasses Mixture to Improve Digestibility and *In vitro* Metabolite Fermentation

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Abstract

Background and Objective: The use of Rice Straw (RS) directly as a single feed not meet the nutritional needs of livestock. Inhibiting factors of using rice straw as feed for ruminants include the low content of nutrients, problems with digestibility and problems with palatability. To determine the optimal use of molasses in a rice straw Urea Lime Molasses (ULM) mixture, the effects of molasses levels were studied with the aim of improving the nutrient value, rumen metabolite content, dry matter and organic matter digestibility *in vitro*. **Materials and Methods:** The study was conducted using a completely randomized design with five treatments and four replications. The treatments were A [89% RS, 4% urea (U), 2% lime (L), 5% molasses (M)], B [84% RS, 4% U, 2% L, 10% M], C [79% RS, 4% U, 2% L, 15% M], D [74% RS, 4% U, 2% L, 20% M] and E [69% RS, 4% U, 2% L, 25% M]. The variables observed were the nutrient value, fermentation metabolite level, Rumen Dry Matter Digestibility (RDMD), Rumen Organic Matter Digestibility (ROMD), *In vitro* Dry Matter Digestibility (IDMD) and *in vitro* Organic Matter Digestibility (IOMD). **Results:** Adding a urea lime molasses mixture increased the nutrient value of RS in proportion to the increase in molasses usage, especially considering Crude Protein (CP). The addition of the ULM mixture to RS could increase the CP content. Similarly, the positive effects of adding the ULM to RS can be seen in metabolite fermentation, based on NH_3 levels. Low levels of NH_3 are due to its high utilization by rumen microbes. The IDMD and IOMD were highest in treatment E. **Conclusion:** The use of molasses at a concentration of 25% is the best when considering the optimal nutrient content, rumen metabolites, IDMD and IOMD.

Key words: Rice straw, urea lime molasses, nutrient digestibility, livestock, crude protein

Received: January 09, 2018 Accepted: August 24, 2018 Published: November 15, 2018

Citation: I.G.L.O. Cakra, A.A.A.S. Trisnadewi, I.M. Mudita and A.A.P.P. Wibawa, 2018. Using Molasses in a Rice Straw Urea Lime Molasses Mixture to Improve Digestibility and *In vitro* Metabolite Fermentation. Pak. J. Nutr., 17: 677-682.

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

An important thing to consider in developing goat farms is the availability of feeds that meet the qualitative and quantitative needs of the goat. The availability of forage for cattle is increasingly limited by the narrowing of agricultural land and plantations. Rice straw (*Oryza sativa*) is an agricultural waste that is a potential alternative feed source instead of forage. Based on the total area of food crops in Indonesia, Indonesia's rice production in 2016 was 75,500,000 t[1]. Each kilogram of harvested rice produces 0.8-1 kg of rice straw[2,3]. In 2016, Indonesia produced approximately 60,400,000-75,500,000 t of rice straw based on dry matter. Assuming that one Animal Unit (AU) is equivalent to a cow weighing 325 kg and consuming 2% of its body weight[4], the available rice straw can supply 9,292,307-11,615,385 AU.

Utilization of rice straw directly as a single feed can-not meet the nutritional needs of livestock. Inhibiting factors of using rice straw as ruminant feed include the low nutrient content of the straw and problems with, digestibility and palatability. The protein content of rice straw varies from 3- 5%[5]. Rice crops harvested in late maturation have high cell wall content and high lignification levels, making the straw difficult for rumen microbes to degrade[6]. Increasing the role of microbes in the provision of nutrients, especially ammonia, may improve the digestibility of rice straw. Supplementation of urea can be used as a source of ammonia (nitrogen); however, urea rapidly releases nitrogen (N) in to the rumen and rapidly produces ammonia thus excessive doses of urea would cause ammonia poisoning and even death in livestock[7]. Huntington *et al.*[8] reported that urea was rapidly hydrolyzed in the rumen and that the peak production of ammonia was achieved at 1 h after offering urea. Techniques for slowing the release of ammonia from urea hydrolysis in the rumen are considered to be more efficient and safe because they can prevent ammonia toxicity[9].

The use of urea in the ration needs to be accompanied by the use of an energy source (carbohydrate source) that is easily soluble or available in the rumen, because synthesizing the optimal microbial protein requires a balance of energy (volatile fatty acids) and nitrogen in N-NH_3 form. Molasses is a feedstuff that is commonly used as a carbohydrate source. A study of the effect of molasses levels in a rice straw urea lime molasses mixture was conducted to determine the optimal level of molasses with respect to nutrient value, rumen metabolite content and dry matter and organic matter digestibility *in vitro*.

MATERIALS AND METHODS

Material: The materials needed for making the rice straw urea lime molasses mixture are sun-dried rice straw, urea, lime and molasses. The equipment used was a chopper, a tarpaulin and a sprayer for spraying the urea lime molasses mixture above the sun-dried rice straw.

Research location: This study was conducted over a 3 month period at the Nutrition and Feed Laboratory, Faculty of Animal Husbandry, Udayana University and Research Center of Livestock Ciawi Bogor, West Java.

Preparation of rice straw urea lime molasses: The mixture of urea lime molasses consists of 4% urea, 2% lime and molasses one of the following levels 5, 10, 15, 20, or 25%. The mixture of urea lime molasses, was prepared according to the treatment, splashed on the dried rice straw, was cut into pieces at a length of approximately 3 cm and spread to 5 cm thickness.

***In vitro* fermentation:** *In vitro* fermentation was performed according to the methods of Minson and McLeod[10]. The steps are as follows; the milled sample of 0.25 g is put into an *in vitro* tube and supplemented with 25 mL of rumen fluid and McDougall's buffer before being incubated in a shaker bath at 40°C for four h. After four hours of incubation, the substrate was centrifuged at 3500 rpm for 10 min. The substrate was separated into precipitate at the bottom and a clear supernatant at the top. This supernatant was sampled for total pH, NH_3 and Volatile Fatty Acid (VFA) measurements. The precipitate was used for the analysis of the digestibility of dry matter and organic matter. The residue of the precipitate was transferred into a vessel with known empty weight, evaporated and dried in a forced-drought oven at 70°C for approximately 12 h and then at 105°C for 9 h before being cooled in a desiccator and then weighed. The mixture was burned into the furnace until the weight of ash was obtained. The blanks were fermented to obtain residues without substrate.

Measurement of (DMD) and (OMD)[11]: Coefficient of DMD and OMD was calculated as follow:

$$\text{DMD (\%)} = \frac{\text{DM sample (g)} - \text{DM residue (g)} - \text{DM blanko (g)}}{\text{DM sample (g)}} \times 100$$

$$\text{OMD (\%)} = \frac{\text{OM sample (g)} - \text{OM residue (g)} - \text{OM blanko (g)}}{\text{OM sample (g)}} \times 100$$

Measurement of NH_3 concentration (phenol hypochlorite method): N-NH_3 levels were determined by the phenol hypochlorite method using a spectrophotometer reading[12]. A total of 15 mL of supernatant was put into a bottle containing 5 drops of concentrated sulfuric acid, which was then diluted 100 times. A total of 5 mL of the diluted supernatant was inserted into a spectro-filled tube with standard solution which was supplemented with 0.2 mL of the phenol solution, 0.2 mL of the nitroprusside solution and 0.5 mL of the oxidizing solution. Color reaction readings with the spectrophotometer were performed 5 min after the addition of the oxidizing solution.

Measurement of VFA total concentration: (steam distillation method)[13]: A total of 5 mL of supernatant is fed into a heated vapor distillation tube. The tube was immediately closed after adding 1 mL of 15% H_2SO_4. The distillation tube is connected to a flask containing boiling water and is heated continuously during the distillation process. The hot vapor will push the VFA through a condensed cooling condenser and accommodated with an erlenmeyer containing 5 mL of NaOH 0.5 N to a volume of about 300 mL, plus a 2-3 drop of phenolphthalein and titrated with HCl 0.5 N. The test is reached the color change from pink to clear or colorless. There was also a blank titration of 5 mL H_2SO_4.

Calculation:

- Total VFA production was calculated as follows :

$$\text{VFA total (mM)} = \frac{(a-b)\times \text{N HCl}\times 1000/5\,\text{mM}}{\text{Sample (g)}\times \text{DM sample (\%)}}$$

Where

a = volume of sample titration
b = volume of blank titration

- A partial VFA Level measurement is determined using Gas Chromatography

Experimental design: In this study, a completely randomized design with five treatment levels of molasses (5, 10, 15, 20 and 25%) was tested and repeated 5 times. The five treatment rations were A (rice straw 89, urea 4, lime 2 and molasses 5%), B (rice straw 84, urea 4, lime 2 and molasses 10%), C (rice straw 79, urea 4, lime 2 and molasses 15%), D (rice straw 74, urea 4, lime 2 and molasses 20%) and E (rice straw 69, urea 4, lime 2 and molasses 25%).

Variables observed: A chemical analysis of the nutrient content from the mixture of rice straw urea lime molasses was conducted. *In vitro* studies observed pH, NH_3 level, total VFA and partial VFA (acetic, propionate and butyric), dry matter digestibility and organic matter digestibility.

Data analysis: The data obtained were analyzed by one-way analysis of variance. If there was a significant difference between treatment ($p<0.05$), Duncan's multiple range test was conducted[14].

RESULTS AND DISCUSSION

The results showed that the nutrient value of rice straw increased proportionally to the level of molasses in the mixture of urea lime molasses added to the rice straw, especially the protein content. The protein content in treatments A, B, C, D and E were 12.01, 12.45, 12.60, 12.69 and 12.81% DM, respectively (Table 1). These findings suggest that the addition of a mixture of urea lime molasses to the rice straw may increase the crude protein content. This indicates that urea when mixed with lime and molasses is still attached to the straw after drying. The protein content in this study was higher than those studies which obtained protein content (8.11%) of dry matter from a urea molasses mixture[15]. This difference in this study is due to adding lime to a mixture of rice straw urea lime molasses to increase the total urea bound to lime.

Table 3 reveals that the decreasing of NH_3 levels in treatment A were due to high utilization of molasses by rumen microbes which can be used as an energy source for microbial growth[16]. These results indicated, the effect of interaction between protein sources and easily fermentable carbohydrate sources on the NH_3 levels.

The high growth of the microbes can be seen from the increasing levels of rumen and in vitro digestibility, where the rumen dry matter and organic matter digestibility was significantly higher in treatment E than in treatment A.

Statistical analyses revealed that the average Dry Matter Rumen Digestibility (DMRD) in treatment A was significantly lower ($p<0.05$) than those of the treatments C, D and E but was not significantly different from treatment B (Table 2). These findings indicated that the addition of urea lime molasses could increase the rumen microbial population, thereby producing the enzymes needed in the fermentation of rumen feed in vitro. The DMRD in treatments A, B, C and D increased, which shows that the increase of molasses from 5-20% could increase the energy readily available for microbial

Table 1: Composition of feedstuff and nutrient content of ration treatment

		Composition (%)				
Feedstuff	Dry matter (%)	A	B	C	D	E
Rice straw	86	89	84	79	74	69
Molasses	82	5	10	15	20	25
Lime	95	2	2	2	2	2
Urea	100	4	4	4	4	4
Nutrient content (%)						
Dry matter		89.30	89.10	88.51	88.40	88.19
Ash (%)		29.17	27.70	26.11	25.76	24.49
Organic matter (DM%)		70.83	72.30	73.89	74.24	75.51
Crude fiber (DM%)		19.22	18.99	17.91	15.61	13.37
Ether extract (DM%)		1.06	1.15	1.53	1.52	1.78
Crude protein (DM%)		12.01	12.45	12.60	12.69	12.81
NFE (DM%)		26.27	29.87	30.53	31.17	35.96

Treatment A: Rice straw 89, urea 4, lime 2 and molasses 5%, Treatment B: Rice straw 84, urea 4, lime 2 and molasses 10%, Treatment C: Rice straw 79, urea 4, lime 2 and molasses 15%, Treatment D: Rice straw 74, urea 4, lime 2 and molasses 20%, Treatment E: Rice straw 69, urea 4, lime 2 and molasses 25%

Table 2: The effect of the use of molasses in the rice straw urea lime molasses mixture on *in vitro* digestibility

	Treatments					
Variables (%)	A	B	C	D	E	SEM
Dry matter rumen digestibility	26.82^{c}	29.70^{bc}	33.77^{b}	43.85^{a}	43.5^{a}	2.0718
Organic matter rumen digestibility	32.52^{b}	34.35^{b}	39.49^{b}	51.06^{a}	50.92^{a}	2.4819
In vitro dry matter digestibility	28.8^{c}	33.35^{b}	44.00^{b}	45.37^{b}	54.25^{a}	1.6763
In vitro organic matter digestibility	36.0^{c}	42.5^{c}	53.8^{ab}	53.21^{b}	59.6^{a}	2.2649

Table 3: The effect of the use of molasses in the rice straw urea lime molasses mixture on *in vitro* fermented products

	Treatments					
Variables	A	B	C	D	E	SEM
NH_3 (mM)	11.22^{a}	10.92^{a}	10.56^{a}	9.99^{a}	7.92^{b}	0.3286
pH	6.99^{bc}	6.97^{c}	7.02^{ab}	7.04^{a}	7.02^{ab}	1.1236
VFA total (mM)	42.37^{c}	45.88^{ab}	47.65^{b}	50.89^{b}	58.12^{a}	1.6572
Acetic acid (mM)	13.14^{b}	13.79^{b}	14.10^{b}	15.41^{a}	15.28^{a}	0.5245
Propionic acid (mM)	2.76^{c}	2.96^{c}	3.53^{bc}	3.94^{ab}	4.74^{a}	0.2880
Butyric acid (mM)	0.26^{c}	0.38^{bc}	0.36^{bc}	0.43^{ab}	0.57^{a}	0.0088
Acetic propionic ratio	4.76^{a}	4.16^{a}	4.09^{a}	3.94^{a}	2.68^{b}	0.439

growth to increase the rumen fermentation *in vitro*. The Organic Matter Rumen Digestibility (OMRD) values in treatments A, B, C, D and E were 32.52, 34.35, 39.49, 51.06 and 50.92%, respectively. Based on an analysis of variance, the OMRD in treatments A, B and C were not significantly different ($p<0.05$) and the OMRD in treatment D was not significantly different from the OMRD in treatment E. However, the OMRD in treatments A, B and C was significantly lower ($p<0.05$) than the OMRD in treatments D and E. This difference is due to the higher molasses use in treatments D and E.

The average *In vitro* Dry Matter Digestibility (IDMD) in treatments A, B, C, D and E was increase. An analysis of variance found that the IDMD in treatment E ($p<0.05$) was highest. The addition of molasses may be able to increase the rumen DMD so that when followed by digestion of the enzyme in vitro, the IDMD will also increase. Similarly, *in vitro* OMD followed the same pattern as DMD because the organic matter is part of the dry matter.

The average NH_3 levels in vitro in treatments A, B, C, D and E were 11.22, 10.92, 10.56, 9.99 and 7.92 mM, respectively. The NH_3 level, in treatment E ($p<0.05$) was lower than that of the treatments A, B, C and D, although the difference was not significant ($p>0.05$). Low NH_3 levels in treatment E showed an increased use of NH_3 by rumen microbes as a result of the availability of molasses as a soluble carbohydrates. Lee *et al.*[17], reported that NH_3 decreased as a result of the addition of fermented carbohydrates. The addition of molasses aims to balance the provision of NH_3 for microbial protein synthesis so as to reduce the presence of NH_3 in the rumen.

The average pH value in treatment D was the highest and was related to the increased use of molasses, from 5-25%. This pH value follows the pattern of the low ratio of NH_3 to VFA, where there is an increase in pH according to the pattern of the addition of molasses. This fact is in accordance with the results of research demonstrating that the NH_3:VFA ratio

without the addition of molasses was higher than the NH_3: VFA ratio with the addition of a molasses treatment of 6 or 12%[18].

Table 3 shows that VFA levels in treatments A, B, C, D and E increased and were highest in treatment E, which, may be due to the presence of 25% molasses in treatment E, because this molasses is an easily fermentable carbohydrate producing VFA. The VFA are the result of carbohydrate metabolism in the rumen.

An analysis of variance revealed that the average concentration of acetic acid among treatments A, B and C was not significantly different but the acetic acid concentrations in treatments D and E are significantly higher than those in the other treatments (Table 3). The increased concentration of acetic acid showed that more fiber digestion occurred in treatments D and E. This dynamic was also indicated by the results of DMD and OMD in treatments D and E, which were significantly higher than those in other treatments.

Table 3 shows that the concentrations of propionic acid in treatments A, B and C were not significantly different but the concentration of propionic acid in treatments D and E was significantly higher compared with that in treatments A and B. The addition of molasses would produce higher propionic acid because molasses is a soluble carbohydrate that can provide a source of energy for microbes and thus improve the digestibility of both dry matter and organic matter (Fig. 1).

Table 3 reveals that the concentrations of butyric acid in treatments A, B and C were not significantly different but the concentrations of butyric acid in treatments E and D, although not significantly different from each other, were significantly higher from concentrations in treatments A, B and C. This dynamic indicates that the increased use of molasses in treatments D and E yields higher butyric acid as a result of fermentative digestion by microbes (Fig. 1). The low levels of NH_3 was due to the availability of soluble carbohydrates as a source of energy. Microbes consumed higher NH_3, therefore, the remaining NH_3 concentration becomes the least in treatment E (Table 3).

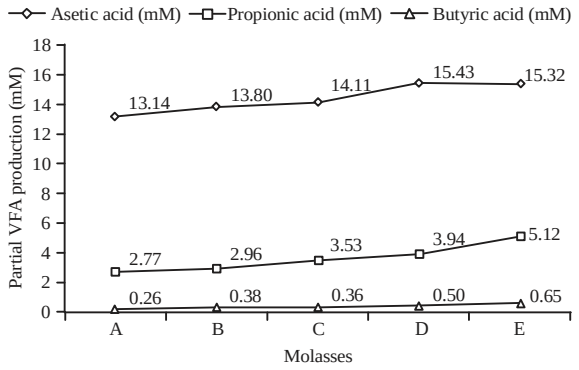

Fig. 1: Partial volatile fatty acid production (mM),
A: 5% molasses, B: 10% molasses, C: 15% molasses, D: 20% molasses and E: 25% molasses

The addition of molasses (20-25%) in the mixture of urea lime molasses can improve the digestibility of dry matter and organic matter compared with other treatments. The use of 25% molasses in the mixture of urea lime molasses gives the highest total VFA fermentation product, acetic, propionic and butyric acid concentrations compared to the other molasses levels.

CONCLUSION

Based on the results of this study, the addition of molasses is optimal at a concentration of 25% when considering the nutrient content, rumen metabolite, dry matter and organic matter digestibility *in vitro*.

SIGNIFICANCE STATEMENT

This study found that the nutritional value of rice straw can be increased with the mixture of urea lime molasses consists of 20-25% molasses, 4% urea and 2% lime, through splashed on the dried rice straw. The optimal molasses concentration is 25% gives the highest nutrient content, rumen metabolite, dry matter and organic matter digestibility *in vitro*.

ACKNOWLEDGMENTS

This study was fully funded by the Institute of Research and Community Service Udayana University, an opportunity for which the authors express gratitude.

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